Method for characterizing nucleic acid fragments

The invention concerns a method for characterizing nucleic acid fragments.

WO-A-99/29897 discloses a method and a kit for identifying nucleic acids. Here, probes of distinguishable mass are used.

US-A 5,929,208 describes a hybridizing assay, wherein the nucleic acid to be characterized is contacted by an oligomer array surface, whereby complementary oligomers of the array hybridize with the DNA to be characterized and non-hybridized nucleic acids are removed. Here, specific positions in an array are provided with charges, so that the binding of oligomers is controlled at specific sites.

Different methods are known at the present time, by which oligonucleotide arrays can be produced. They can be roughly classified into 3 groups:

1) All oligomers are produced individually and in relatively large quantities in the conventional manner in the test tube or in special automatic synthesis devices and then individually pipetted onto the carrier. For this purpose, usually automatic, highly-precise micropipetting robots are used. The advantage of this method is that it is based for the most part on standard methods and devices that have already been optimized. Qualitatively superior DNA arrays with very pure oligomers can be produced in this way, which has an extremely positive influence on the detection sensitivity and reliability that can be obtained with the array. The great disadvantage of the method is that it is very time-consuming and is

thus expensive. This applies particularly to the synthesis of the individual oligomers.

The purchase of ready-to-use oligomers does not represent a solution, since commercially synthesized oligonucleotides in large quantities are also extremely expensive, because the synthesis of the monomers is relatively expensive, patent fees must be paid for several special designs, and also the solvents used are expensive due to their required purity.

2) The oligomers are synthesized by pipetting minimal quantities directly onto the substrate. The oligomer chain provided therein is constructed, nucleobase by nucleobase, at each grid point. For pipetting, as in method (1) a specialized micropipetting robot device is similarly used, or, e.g., a device that contains channels for introducing the individual synthesis building blocks to the respective points of the array (EP-A 0915897). The chemical synthesis method is basically the same as for conventional oligomer synthesis in automated synthesis equipment. The difference is that all oligomers are prepared simultaneously, independent of their number, by a single automatic unit, directly at the provided determination site. The separate operating steps of oligomer synthesis and micropipetting as in method (1) are now combined into a single operating step. The expense for equipment and for manual labor is considerably reduced when compared with method (1).

3) The oligomers, as in method (2), are synthesized directly on the substrate, but the targeted binding of the correct nucleobases to the correct grid points is accomplished by a completely parallel photolithographic technique

originating from semiconductor manufacture, instead of sequential, precisely targeted pipetting steps. The method is based on the fact that 5'-OH protective groups can be removed from oligonucleotides in a targeted manner with light of a specific wavelength. By suitable local irradiation patterns, oligonucleotide ends can thus be made reactive at precisely those grid points at which it is desired that a new nucleotide building block will bind in the next step. When the array surface is completely wetted with a nucleotide building-block solution, a nucleobase will thus be bound only at the previously exposed sites, and all of the unexposed sites will remain unchanged. The local exposure patterns are produced by positioning a photomicrograph black-and-white mask between the substrate and the light source, which covers all grid sites which will not be made reactive. The lengthening of the oligomer chains on all grid points by one nucleobase is accordingly accomplished as follows: by means of a first mask, just those grid points are exposed, which must be extended by the first of the 4 possible types of nucleobases (e.g., C). Then the array is wetted with a solution of the respective nucleotide base, whereupon only the exposed points are lengthened by this base. Since the newly bound nucleotide building blocks are still all made available by means of a protective group, they can no longer react in the following steps until their protective groups are cleaved by another exposure. After this reaction step, the array is washed. Now, by means of a second mask, just those grid sites are exposed, which must be extended by the second of the 4 possible nucleobases (e.g., T). The array is again wetted with a solution of the corresponding nucleotide building block and the exposed sites are lengthened by

this base. The procedure is conducted in the same way for the other two nucleobases (G and A). Consequently, four exposure steps and thus 4 photomasks are required for the lengthening of all oligomers by one nucleobase.

Due to the high parallel nature in processing, this method is very rapid and efficient, and it is also well suitable for the purpose of achieving very high grid densities, due to the high precision that can be obtained with photolithography.

An overview of the prior art relative to oligomer array production can also be taken from a special publication that appeared in January 1999 of Nature Genetics (Nature Genetics Supplement, Volume 21, January 1999) and the literature cited therein.

Patents, which generally relate to the use of oligomer arrays and photolithographic mask designs, include, e.g., US-A 5,837,832; US-A 5,856,174; WO-A 98/27430 and US-A 5,856,101. There also exist several material and method patents, which limit the use of photolabile protective groups to nucleosides, thus, e.g., WO-A 98/39348 and US-A 5,763,599.

Various methods exist for immobilizing DNA. The best-known method is the solid binding of a DNA, which is functionalized with biotin, to a strepavidin-coated surface. The binding strength of this system corresponds to a covalent chemical bond without being one. In order to be able to bind a target DNA covalently to a chemically prepared surface, the target DNA needs to have the corresponding functionality. DNA itself does not have a functionalization that is suitable. There are different methods for introducing a suitable functionalization into a target DNA: two functionalizations that are easy to manipulate are primary

aliphatic amines and thiols. Such amines are converted quantitatively with N-hydroxysuccinimide esters and thiols react quantitatively with alkyl iodides under suitable conditions. The one difficulty is how to introduce such a functionalization into a DNA. The simplest method is the introduction by a primer of a PCR. The indicated methods utilize 5'-modified primers (NH_2 and SH) and a bifunctional linker. An essential component of the immobilization onto a surface is the condition of the surface. Systems described up to the present time primarily comprise silicon or metal (magnetic beads). Another method for binding a target DNA is based on using a short recognition sequence (e.g., 20 bases) in the target DNA for hybridizing to a surface-immobilized oligonucleotide.

Various enzymatic methods have also been described for introducing chemically activated positions into a target DNA. Here, a 5'-NH_2 functionalization is introduced enzymatically into a target DNA. 5-Methylcytosine is the most frequent covalently modified base in the DNA of eukaryotic cells. For example, it plays a role in the regulation of transcription, genomic imprinting and in tumorigenesis. The identification of 5-methylcytosine as a component of genetic information is thus of considerable interest. 5-Methylcytosine positions, however, cannot be identified by sequencing, since 5-methylcytosine has the same base pairing behavior as cytosine. In addition, in the case of a PCR amplification, the epigenetic information that is borne by 5-methylcytosines is completely lost.

Several methods are known for solving these problems. For the most part, a chemical reaction or enzymatic treatment of genomic DNA is conducted, as a consequence of which, cytosine can be distinguished from methylcytosine

bases. A current method is the reaction of genomic DNA with bisulfite, which leads to a conversion of cytosine bases to uracil after alkaline hydrolysis in two steps (Shapiro, R., Cohen, B., Servis, R. Nature 227, 1047 (1970)). 5-Methylcytosine remains unchanged under these conditions. The conversion of C to U leads to a change in the base sequence, from which the original 5-methylcytosines can now be determined by sequencing (only methylcytosines can still provide a band in the C lane).

An overview of the other known possibilities for detecting 5-methylcytosines can be taken from the following review article together with all of the references cited therein: Rein, T., DePamphilis, M. L., Zorbas, H., Nucleic Acids Res. 26, 2255 (1998).

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI) is a new, very high-performing development for the analysis of biomolecules (Karas, M. and Hillenkamp, F. 1998. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal. Chem. 60: 2299-2301). An analyte molecule is embedded in a matrix absorbing in the UV. The matrix is vaporized in vacuum by a short laser pulse and the analyte is transported unfragmented into the gas phase. An applied voltage accelerates the ions in a field-free flight tube. Based on their different masses, ions are accelerated to differing degrees. Smaller ions reach the detector earlier than larger ions. The time-of-flight is converted into the mass of the ions. Technical innovations of hardware have significantly improved the method. Delayed extraction (DE) is worthy of mention. For DE, the acceleration voltage is turned on with a delay

relative to the laser pulse and in this way an improved resolution of the signals is achieved, since the number of collisions is reduced.

MALDI is excellently suitable for the analysis of peptides and proteins. For nucleic acids, the sensitivity is approximately 100 times poorer than for peptides and decreases overproportionally with increasing fragment size. The reason for this lies in the fact that only a single proton must be captured for the ionization of peptides and proteins. For nucleic acids, which have a backbone with multiple negative charges, the ionization process is basically inefficient due to the matrix. For MALDI, the selection of the matrix plays an extremely important role. Several very high-performance matrices have been found for the desorption of peptides, which produce a very fine crystallization. In fact, several high-performance matrices have in the meantime been found for DNA, but the difference in sensitivity was not reduced in this way. The difference in sensitivity can be reduced by modifying DNA chemically in such a way that it is similar to a peptide. Phosphorothioate nucleic acids, in which the usual phosphates of the backbone are substituted by thiophosphates, can be converted into charge-neutral DNA by simple alkylation chemistry.

The coupling of a "charge tag" to this modified DNA results in an increase in sensitivity to the same range as is found for peptides. The possibility of utilizing matrices that are similar to those used for the desorption of peptides is offered by these modifications. Another advantage of charge tagging is the increased stability of the analysis against contaminations that greatly hinder the detection of unmodified substrates. PNAs and methylphosphonate

oligonucleotides have been investigated with MALDI and thus can be analyzed in this way.

An array with many thousand target DNAs can be immobilized on a solid phase and then all target DNAs can be investigated jointly for the presence of a sequence by means of a probe (nucleic acid with complementary sequence).

A correspondence of the target DNA with the probe is obtained by a hybridization of the two segments with one another. Probes can be any nucleic acid sequences of any length. Different methods exist for the selection of optimal libraries of probe sequences, which minimally overlap with one another.

Probe sequences can also be combined in a targeted manner in order find specified target DNA sequences. Oligofingerprinting is one approach, in which this technology is applied. A library of target DNAs is scanned with short nucleic acid probes. For the most part, the probes here are only 8-12 bases long. Each probe is hybridized once to a target DNA library that has been immobilized on a nylon membrane. The probe is radioactively labeled and the hybridization is evaluated on the basis of localizing the radioactivity.

The utilization of oligomer probe libraries for identifying immobilized nucleic acids by means of mass spectrometry has been described (EP 97 12 1471.3 and EP 97 12 1470.5). Of course, no oligomer arrays are used in this method.

Probes with multiple fluorescent labels have been used for the scanning of an immobilized DNA array. The simple introduction of Cy3 and Cy5 dyes to the 5'OH of the respective probe has been particularly suitable for fluorescence

labeling. The detection of fluorescence of the hybridized probes is made, for example, by means of a confocal microscope. The dyes Cy3 and Cy5, in addition to many others, are commercially available.

The object of the present invention is to make available a method, which overcomes the disadvantages of the prior art.

According to the invention, a method is created for characterizing a nucleic acid fragment, whereby the following method steps are conducted:

a) a nucleic acid fragment to be characterized is immobilized on a surface;

b) an array of oligomers is prepared on a second surface, wherein the oligomers are provided with a label;

c) the synthesized oligomers are stripped from the surface without leaving a pregiven region on the surface;

d) the surface on which the nucleic acid to be characterized is immobilized is contacted with the oligomer array surface, whereby complementary oligomers of the array hybridize to the DNA to be characterized;

e) non-complementary oligomers are removed;

f) the complementary oligomers are detected by means of their label, whereby sequence information is determined on the basis of the site on the surface.

It is preferred according to the invention that the nucleic acid fragment to be characterized is an amplified product of genomic DNA.

A particularly preferred form of embodiment of the method of the invention provides that the genomic DNA is reacted with a solution of a bisulfite, disulfite or hydrogen sulfite prior to the amplification.

It is further preferred that the nucleic acid fragment to be characterized is covalently bound to the surface. It is also preferred that an amino function is introduced into the nucleic acid fragment to be characterized and this function binds to a glass surface that has been derivatized by silanizing.

The method wherein the oligomers of the array are bound covalently to the second surface is also preferred according to the invention. It is also preferred that an amino function is introduced into the oligomers of the array and this function binds to a glass surface derivatized by silanizing.

It is also preferred that the oligomer array is produced by solid-phase synthesis of the oligomers on the second surface.

It is particularly preferred that the solid-phase synthesis of the oligonucleotides on the second surface is conducted in a closed synthesis chamber, in which the synthesis reagents are introduced selectively. It is particularly preferred here that the synthesis of the oligomers is conducted by selective introduction of the synthesis reagents at the respective sites at which the oligomers are synthesized.

It is further preferred according to the invention that photolithographic methods and photolabile protective groups are used for the oligomer synthesis.

In addition, it is also preferred that electronically controllable and/or changeable masks are used for the photolithographic process. Here, it is

particularly preferred that a mirror array that can be selectively turned on is used for producing an exposure pattern for the photolithographic process.

It is further preferred according to the invention that the synthesis of oligomers is conducted in an array of cavities, which are also used, if needed, as chambers for the hybridization.

A preferred variant of the method according to the invention is also that the nucleic acid fragments to be characterized are immobilized on an array of cavities, which are used also, if needed, as chambers for the hybridization.

It is preferred that chemical groups that effect a change in mass and/or fluorescence are used as the labels of the oligomers. In particular, It is preferred in the method of the invention that the hybridized oligomers are detected by means of mass spectrometry, preferably by means of matrix-assisted laser desorption/ionization mass spectrometry (MALDI).

Another subject of the present invention is a kit for conducting the method according to one of the preceding claims, comprising reagents and/or reference nucleic acid fragments and/or reference DNA and/or treated surfaces and/or photolithographic masks and/or oligomers.

The present invention thus describes a method for characterizing nucleic acids. In a particularly preferred variant, it serves for identifying cytosine methylation patterns in amplified products of genomic DNA.

The invention thus concerns a method for the characterizing of nucleic acids. For this purpose, the nucleic acid is immobilized on a surface. An array of labeled oligomers, preferably oligonucleotides, is prepared on a second surface

by means of solid-phase synthesis, and then the oligomers are cleaved from the surface, without the oligomers leaving a pregiven region on the second surface. The two surfaces are contacted after the addition of a small quantity of a buffer solution and the synthesized oligomers hybridize at those sites at which their synthesis also has occurred, to the nucleic acids immobilized on the first surface. At those sites at which a hybridization has occurred, the oligomers can subsequently be recorded by means of their label. The pattern of hybridized oligomers, which is obtained in this way, is used for determining sequence information in the nucleic acid to be characterized. In a particularly preferred variant, the method serves for identifying cytosine methylation patterns in genomic DNA. For this purpose, the latter is treated and amplified with a bisulfite solution before it is introduced onto the first surface.

For the characterizing of an amplified genomic DNA sample, oligomer arrays are used, in which the DNA sample is fluorescently labeled and is hybridized to such an array of immobilized oligomers (prior art). It is also possible to prepare an array of amplified products and to hybridize probes thereto. However, only a few probes can be used simultaneously for this purpose, since they can differ only in a limited manner, due to, e.g., their fluorescence. A patent that deals with this problem by means of mass spectrometry is mentioned in the prior art.

This invention describes the inverse variant, of immobilizing the DNA to be identified on the surface, and nevertheless to utilize the advantages of oligomer array technology. It offers numerous advantages. First of all, the hybridization

can be conducted substantially more rapidly, since in the variant described here, short oligonucleotides can diffuse over an extremely short distance to the immobilized target DNA and these can also be present in a large excess. Secondly, the chip with the costly immobilized target DNA can be reused as frequently as necessary for its complete characterization after the removal of the oligomers. It is also in many cases simpler to introduce labels on the oligomers rather than on the amplified DNA which is present frequently in substantialy smaller quantities. This is true particularly if detection is produced by means of mass spectrometry, since in this case, the oligomers can be detected directly. This variant also permits the use of oligomer libraries, whose components can all be distinguished by means of their mass.

Another substantial advantage of the present invention is that the oligomers no longer necessarily hybridize to the target DNA in the direct vicinity of the surface, as is otherwise necessarily the case in arrays of immobilized oligomers. This frequently causes problems, particularly with respect to hybridizing efficiency. However, the general advantages of oligomer-array synthesis, as described in the prior art, remain in the case of the present invention.

The method according to the invention for characterizing nucleic acids is comprised of the following steps:

1. A nucleic acid to be characterized is immobilized on a surface.

2. An array of oligomers, which are provided with a detectable label, is prepared on a second surface.

3. The synthesized oligomers are stripped from the surface, without leaving a pregiven region on the surface.

4. The surface on which the nucleic acid to be characterized has been immobilized is contacted with the oligomer-array surface.

5. Complementary oligomers of the array hybridize to the DNA to be characterized and non-complementary oligomers are removed by washing steps.

6. The complementary oligomers are detected by means of their label and sequence information is determined on the basis of their site on the surface.

The nucleic acid to be characterized can be genomic DNA, preferably amplified fragments of a genomic DNA sample, or also RNA. In a particularly preferred variant of the method, the genomic DNA sample can be pretreated with a bisulfite solution in order to effect a conversion of cytosine to uracil, while methylcytosine is not converted under these conditions. It is possible by means of the preceding step to use the method for the identification of methylation patterns in genomic DNA. The genomic DNA sample is preferably amplified in order to improve the sensitivity of the method. This can be produced in a particular preferred manner by means of PCR.

In the first step of the method, the DNA to be characterized is now immobilized on a surface. This surface may be comprised of glass, quartz glass, or also, for example, silicon. In a preferred variant of the method, the surface is chemically activated, so that one or more covalent bonds of the nucleic acid to be characterized can be created with the functionalized surface. This activation of the surface is preferably conducted by silanization. First of all, an activation with

epoxy functions is considered, to which the DNA to be characterized either binds directly or, however, preferably by means of a primary amino function introduced in the PCR by means of a primer. Secondly, the surface can be amino-functionalized by means of silanization. The DNA to be characterized may then be coupled with this amino function by means of a linker molecule. The DNA to be characterized in this case preferably bears amino or mercapto functions preferably added in the PCR. For example, SIAB, DMS or PITC are used as bifunctional linker molecules.

With respect to the pretreatment of the surface and the linker procedure, the same holds true for the second step of the method, the preparation of the oligomer arrays on the second surface. The oligomer arrays are efficiently produced, as in the prior art, but with the difference that they can be cleaved again from the solid phase. This cleavage can be produced either photochemically or by the action of an acid or base, preferably a base. The surface can be derivatized for the production of the oligomer arrays both with primary alcohol or amino functions. The primary alcohol functions are introduced according to the prior art by means of epoxidation of the surface and subsequent reaction with an oligoethylene glycol, but are preferably introduced by the amino functionalization of glass surfaces that has been recently proposed by Beier et al. (Nucleic Acids Research, 1999, pp. 1970-77). In order to be able to conduct a photochemical cleavage, a photocleavable linker must first be preferably introduced after the amino or OH functionalization. In another embodiment of the method, the modified protective group strategy proposed by Beier and Pfleiderer

can be applied for the cleavage of oligomers after synthesis, whereby the base DBU is used for the cleavage of oligomers from the surface (DE-A 196 25 397).

In a particularly preferred variant of the method, the oligomer arrays are synthesized in a closed synthesis chamber, into which synthesis reagents can be selectively introduced. The monomers are guided each time directly to the respective sites of synthesis, preferably by a pin tool or by means of a piezo pipetting robot, whereby the synthesis chamber is opened for this step. Alternatively, synthesis can be conducted by means of photolithographic methods, as also described in the prior art. By means of a mask, light is conducted only to those regions in which a chain lengthening of the oligomer is to be produced by one specific monomer each time (see the prior art). The use of dynamic masks, which can be changed by electronic control, is particularly preferred. These masks can be either an LCD display, a microseal array or a fiberoptics bundle that can be turned on. Also, light can be selectively introduced to points on the surface by means of arrays of tiltable micromirrors, as are used in modern projectors. Alternatively, viscoelastic micromirrors are used. Photolithographic methods require the use of photolabile protective groups on the monomers, whereas conventional protective groups can be used when the monomers are introduced by means of, e.g., a pipetting system.

It is also possible to synthesize several different oligomers or oligomer libraries to one or more sites on the second surface, as long as they can all be distinguished after hybridizing by means of the detection method used.

The oligomer arrays may be comprised of oligonucleotides and/or PNAs (peptide nucleic acids). Little is changed with respect to the principal procedure for synthesis, and the synthesis methods corresponding to the prior art are applied each time. However, if libraries are used at one or more points of the array, it is preferred to use PNA, since the resulting components of the library can be more easily distinguished by mass in this case. In the array synthesis, the oligomers are provided with a labeling, which can be preferably either a fluorescent dye or, however, a specific mass. The mass can be either that of the oligomer itself or, however, that of the oligomer plus a mass labeling in the form of a functional group.

In the third step of the process, the cleavage of the oligomers from the surface is now conducted. Care is to be taken that the cleavage must not lead to a lateral movement of these oligomers on the surface, because the order of the array would be disrupted. In order to avoid this, in a particularly preferred variant of the method, the synthesis of the oligomer array is already conducted in such a way that the synthesis of any one type of oligomer occurs in a cavity assigned to it. The oligomer does not leave this cavity even after its, e.g., photochemical cleavage from surface.

Finally, in the fourth step of the method, the stripped and labeled oligomers that are still present, however, in the original two-dimensional arrangement are hybridized to the nucleic acids to be characterized. This is done in a particularly preferred variant of the method by placing hybridizing buffer in the cavities of the second surface, in which the oligomers are found, and then

the first surface with the nucleic acids to be characterized is contacted with the second surface. Alternatively, the first surface exclusively or both surfaces can also contain cavities, which are then filled each time with hybridizing buffer. This can be done, for example, preferably with a pipetting robot or a pin-tool robot. The grid of cavities preferably corresponds to the grid of oligomers in the array on the second surface.

After hybridizing the complementary oligomers to the nucleic acids to be characterized, the two surfaces are separated from one another and the non-complementary oligomers are removed from the first surface by washing steps.

In the sixth step of the method, the complementary oligomers are detected by means of their labelings. It is known from the array synthesis, which oligomers may have hybridized at which point on the first surface. The labelings are fluorescent dyes in a particularly preferred variant of the method and Cy3 or Cy5 again are particularly preferred. Now, the fluorescence at each site on the surface can be detected in a fluorescence scanner and the identity of the hybridized oligomers can be determined with the spatial information present. Complete or partial sequence information on the DNA to be characterized can in turn be obtained from this.

In a particularly preferred variant of the method, the identity of the hybridized oligomers provides information on cytosine methylations in the DNA to be characterized, if the latter has been pretreated with a bisulfite solution prior to step 1 of this method.

In another preferred variant of the method, the hybridized oligomers are detected by means of mass spectrometry, preferably matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). For this purpose, the first surface with the hybridized oligomers is first coated with a matrix and then the respective points are entered directly in the mass spectrometer. The software of the mass spectrometer permits the assignment of the mass spectra obtained to the respective points on the surface. The hybridized oligomers are clearly identified by means of their masses. In a preferred variant, the oligomers in the array synthesis are provided with mass labels. These involve functional groups, the objective of which is only to increase the mass of the oligomer by a specific amount, in order to reliably distinguish it from oligomers of another sequence. Thus several different oligomers or even oligomer libraries can be synthesized even at one point on the second surface beforehand, and their masses will be reliably distinguished at least one point of the array each time. In a particularly preferred variant of the method, the identity of the hybridized oligomers again provides information on cytosine methylations in the DNA to be characterized, if the latter has been treated with a bisulfite solution prior to step 1 of this method. In a particularly preferred variant of the method, this information is entered into a database and correlated with the phenotype belonging to the DNA to be characterized.

The following examples explain the invention:

Example 1:

Synthesis of DNA with 5'-DMT and photolabile protective groups

DNA oligomers are synthesized according to the established phosphoramidite method, here on modified glass surfaces. Acid-labile DMT groups or photocleavable groups, which are known in and of themselves, may be used as 5' protective groups. For cleaving these groups, in the case of DMT, a solution of trichloroacetic acid in dichloromethane is used, while in the case of the photolabile groups, light with a suitable wavelength, preferably between 320 and 365 nm, is used. The protective groups on the exocyclic amines and the phosphates are cleaved according to standard procedures with concentrated ammonia.

Example 2:

Synthesis of PNA oligomer arrays

PNA oligomers are synthesized (as an oligomer array) by spotting the individual components onto the chip. After spotting a monomer or a reagent, the chip is washed with dimethylformamide, in order to remove unreacted chemicals. Thus, e.g., the PNA sequence 5'-AGC CAG CTC ACT ACC TAG-3' is constructed from the C-terminal (3') up to the N-terminal (5'). Synthesis is produced by binding the acid function of the N-protected monomer G (Fmoc, 5 equivalents) in a DMF solution together with 5 equivalents of DIPEA, 7.5 equivalents of lutidine and 4.5 equivalents of HATU, onto the modified glass surface. The 5'-amino groups of monomers A, C, G and T are protected with an Fmoc group, and the exocyclic amino groups on monomers A, C and G are

protected with a Bhoc group. PNA monomers and chemicals can be obtained commercially (e.g. Perkin Elmer).

Then, washing is conducted with DMF, unreacted NH_2 groups are capped with a mixture of acetanhydride/lutidine in DMF, washed with DMF, and the protected 5'-amino function is deprotected with 20% piperidine in DMF. After washing with DMF, the A monomer is spotted onto the bound G monomer. The complete PNA sequence is synthesized by the subsequent synthesis cycles. The Bhoc groups of the exocyclic amino groups of bases A, C and G are removed at the end of the synthesis with trifluoroacetic acid in the presence of m-cresol.

Example 3:

Synthesis of DNA on chips, which bear a photocleavable linker

In a first step, the glass substrates are chemically modified, so that a targeted binding of oligonucleotide building blocks can occur, as in the prior art. By introducing various types of oligonucleotides onto a substrate, oligonucleotide arrays can be produced in the usual manner. For this purpose, the substrates are silanized, whereby the silane bears a functionalized (for example OH- or NH_2) alkyl chain. Then the surface is provided with a photolabile linker, for example, 4[4-1-(Fmoc-aminomethyl)-2-methoxy-5-nitrophenoxybutanoic acid (Novabiochem). This linker permits a cleavage of the oligonucleotides upon irradiation of light of 365-nm wavelength. The oligonucleotides, for example, the sequence 5'-AGC CAG CTC ACT ACC TAG-3', are synthesized as was

described in Example 1 for DMT on the deprotected amino function of the linker. Protective groups of the exocyclic amines, for example, benzoyl or isobutyryl, are removed with concentrated ammonia. The DNA is cleaved photochemically from the entire chip and is then washed from the surface.

Example 4:

Synthesis of PNA on chips, which bear a photocleavable linker

The modification of the surface of the glass substrate is conducted as described in Example 3 with a spotted photolabile linker. The PNA sequence, for example, 5'-AGC CAG CTC ACT ACC TAG-3', is synthesized by spotting the components as described in Example 2 onto the deprotected amino function of the photolabile linker. The Bhoc protective groups are removed with trifluoroacetic acid and the synthesized PNA strand is cleaved with light of 365-nm wavelength.

The PNA synthesis on photocleavable linkers may also be conducted over a large surface on the entire chip, whereby the components are rinsed over the chip. Deprotection is carried out as described.

Example 5:

Synthesis of DNA on chips, which bear a non-photocleavable linker

In the first step, the glass substrates are modified chemically, so that a targeted binding of oligonucleotide building blocks, which is known in and of itself, can be conducted. For this purpose, the substrates are silanized, whereby

the silane bears a functionalized (for example, OH- or NH_2-) alkyl chain. The non-photocleavable linker is synthesized by coupling 5'-protected T, A, C or G monomers with succinic anhydride and dimethylaminopyridine. For example, conventional DMT groups are used as protective groups on the monomers for a large-surface DNA synthesis or photolabile groups are used in the case of a DNA array. The pretreated monomer is coupled to the substrate and the synthesis, for example, of the sequence 5'-AGC CAG CTC ACT ACC TAG-3', is conducted as described in Example 1. The 5'-protective groups are cleaved either with trichloroacetic acid or by irradiation of light (365 nm). The DNA is dissolved from the substrate by the action of ammonia vapors and the array is transferred to a nitrocellulose membrane by blotting.

Example 6:

Blotting of DNA oligomer arrays

In order to be able to hybridize the oligomer arrays of Example 5 which were cleaved from the modified glass surface to the sample DNA, the arrays are blotted onto nitrocellulose (Amersham Pharmacia Biotech, Hybond series, nitrocellulose on nylon or PVDF), analogously to the established method of "Southern blots". The arrangement of the oligomers in the array is not substantially changed, as long as the distance between the points in the array is sufficient. For this purpose, the glass carrier or slide is placed by its loaded side onto a nitrocellulose substrate, which has been premoistened with a hybridizing buffer (SSC + SDS, Denhardt's reagent). By exercising pressure, there is a transfer of the oligomers to the nitrocellulose. The sample DNA, with which the

oligomers then specifically hybridize, is already immobilized on the nitrocellulose. Non-hybridized oligomers are washed away. Detection is effected by means of a fluorescent label introduced on the oligomers (see Example 9).

Example 7:

Synthesis of PNA on chips, which bear a non-photocleavable linker

The substrate surface is silanized, whereby the silane bears a functionalized (for example, NH_2- or glycidoalkyl) alkyl chain. Then the amine is coupled with a carboxylic acid of a non-photocleavable linker, for example, a protected hydroxyalkyl carboxylic acid or epoxide under acidic conditions with ethylene glycols of different chain lengths. The PNA synthesis, for example, of the sequence 5'-AGC CAG CTC ACT ACC TAG-3' is conducted as described in Example 2. The coupling of monomer G with the terminal alcohol leads to the formation of an ester. The PNA synthesis may also be conducted with a photocleavable protective group on the amino group. Cleavage is produced with light of 365-nm wavelength instead of with 20% piperidine in DMF. The PNA is stripped from the substrate by the action of ammonia vapors and is contacted with the sample DNA by blotting onto nitrocellulose.

Example 8:

Blotting of PNA oligomer arrays

The method for identifying PNA oligomers is similar to that for DNA identification; a PNA hybridizing buffer is used. This method is analogous to the established methods of "Western blots" for proteins.

Example 9:

Coupling of fluorescent dye to PNA oligomers

In order to couple a fluorescent dye, preferably Cy3 or Cy5, to PNA oligomers, the N-terminal (the 5'-amino group) is reacted with the N-hydroxysuccinimide derivative of the dye with the formation of an amide. For this purpose, the dye derivative is dissolved in DMF or DMSO and contacted with the immobilized oligomers in the presence of triethylammonium hydrogen carbonate buffer. After 2 h, the reaction is terminated, and the excess or hydrolyzed dye is removed by washing steps with water and methanol.

Example 10:

Immobilizing of PCR products onto modified glass surfaces

In order to be able to immobilize PCR products onto glass substrates, both the PCR products as well as the glass surface are modified. An aminopropyl derivative, which supplies a free amino group, is coupled to the 3' end of the PCR product. The glass surface is derivatized with 3'-glycidoxyalkyl trimethoxysilane and catalytic quantities of diisopropylethylamine and bears an epoxide on the surface. The immobilization is conducted by spotting a solution of PCR products in 0.1 M potassium hydroxide and incubation for 6 hours at 37°C and 100% air humidity. Then unbound PCR products are removed by washing with water.

Example 11:

Immobilizing the sample DNA on nitrocellulose or PVDF membranes

Sample DNA is immobilized on nitrocellulose or PVDF membranes according to the instructions of the manufacturer. Particularly suitable are membranes which have already been introduced onto a glass surface in a microscope slide format (e.g., of Schleicher and Schüll) and thus can be simply analyzed in conventional fluorescence scanners after the hybridization. After immobilizing, the membranes are moistened with hybridizing buffer.